

IDENTIFICATION AND MUTAGENICITY OF AMINO- AND HYDROXY-SUBSTITUTED
NITROGEN AND SULFUR HETEROCYCLES IN A SOLVENT-REFINED COAL LIQUID

Masaharu Nishioka, Gary M. Booth, and Milton L. Lee

Departments of Chemistry and Zoology
Brigham Young University
Provo, UT 84602

Raymond, N. Castle

Department of Chemistry
University of South Florida
Tampa, FL 33620

INTRODUCTION

Development of coal upgrading processes such as gasification and liquefaction must be continued because of the long-term uncertainty in the availability of petroleum resources. Identification of components and investigation of the environmental effects of coal-derived products produced in these upgrading processes are required. Although numerous studies describing the identification and characterization of polycyclic aromatic hydrocarbons (PAH) and polycyclic aromatic compounds (PAC) containing a single heteroatom have been reported, PAC containing more than one heteroatom have been only tentatively identified until recently. In the last year, many new PAC containing two heteroatoms in a solvent-refined coal liquid were positively identified using capillary column gas chromatography. These compounds include hydroxylated thiophenic compounds (1), aminodibenzothiophenes (2), hydroxylated nitrogen heterocycles (3), azathiophenic compounds (4), and amino-substituted nitrogen heterocycles (5). In this paper, the analytical methods for the identification of trace amounts of PAC containing two heteroatoms in complex coal-derived materials are reviewed. The structural characteristics of the PAC formed in a solvent-refined coal liquid and a coal tar were compared, and standard compounds were synthesized for verification of identifications and for mutagenicity testing.

EXPERIMENTAL

A solvent-refined coal heavy distillate (SRC II HD: 260-450°C boiling point range) was obtained from the Fort Lewis, Washington, pilot plant (operated by the Pittsburg & Midway Coal Mining Co.). A coal tar was obtained from the National Bureau of Standards, Washington, DC. This was a medium crude coke oven tar. Standard compounds were obtained commercially or synthesized by us (6).

The SRC II HD material and coal tar were fractionated into chemical classes by adsorption chromatography on neutral alumina and silicic acid (3, 7). The third (A-3) and fourth (A-4) alumina column fractions which were composed of the nitrogen- and hydroxy-containing PAC, respectively; the second (S-2) and third (S-3) silicic acid column fractions from the A-3 fraction which were composed of the amino- and tertiary nitrogen-containing PAC, respectively; and the second (S-2') silicic acid column fraction from the A-4 fraction which was composed of hydroxy nitrogen-containing PAC were analyzed in this study.

Hewlett-Packard Model 5880 gas chromatographs equipped with flame ionization (FID), sulfur-selective flame photometric (FPD), nitrogen-selective thermionic (NPD), and ^{63}Ni electron capture (ECD) detectors were used in this study. The capillary columns were prepared by coating 10-20 m x 0.2 or 0.3 mm i.d. fused silica tubing (Hewlett-Packard, Avondale, PA) with SE-54, a 25% biphenyl polymethylsiloxane (8,9), or a smectic liquid-crystalline polymethylsiloxane stationary phase (10-13) (0.25 μm film thicknesses). A Hewlett Packard Model 5982A GC-MS system was used to obtain mass spectral confirmation of identified compounds. The Ames mutagenicity assays were performed as described by Ames *et al.* (14) with minor modifications.

RESULTS AND DISCUSSION

The chemical complexities typically associated with coal-derived products require stationary phase selectivity in addition to high efficiency for their analysis by capillary column gas chromatography. We have recently synthesized a new polarizable stationary phase, a biphenyl polysiloxane (8,9), and several liquid-crystalline polysiloxanes (10-13) for the analysis of PAC. The biphenyl polysiloxane provided unique selectivity for the polar amino- and hydroxy-substituted nitrogen and sulfur heterocycles. The polar substituent groups on the PAC interact with the π -electron cloud of the biphenyl group. Blocking of polar groups is sometimes recommended for strongly polar compounds. Blocking of the polar group leads to a decrease in the dipole-induced dipole interactions between solutes and the stationary phase. This "softer" interaction often results in improved resolution. All isomers of the hydroxydibenzothiophenes in the coal tar were resolved and identified using a 25% substituted biphenyl phase (Figure 1) after blocking of the hydroxyl group. In comparison, only the 1- and 2-hydroxydibenzothiophenes were detected in the SRC II HD. Similarly, improved resolution was observed for the pentafluoropropyl (PFP) derivatives of the alkylated aminodibenzothiophenes (2). The large retention differences between the original polar compounds and their derivatives gave complementary evidence for their identification (1,2).

The separations achieved on liquid-crystalline stationary phases is based on the molecular geometries of solutes, such as size, shape, and planarity, in addition to other factors associated with conventional stationary phases. Positive identifications of the hydroxylated thiophenic compounds were achieved using both a smectic liquid-crystalline stationary phase and the 25% biphenyl stationary phase (1). This liquid-crystalline phase was also used for the separation of isomeric PAH (15), sulfur heterocycles (16), and amino-PAH (5).

Approximate concentrations of the amino- and hydroxy-substituted nitrogen and sulfur heterocycles were estimated to be 1-10 ppm in the SRC II HD. In comparison, corresponding PAC containing only one heteroatom were present at approximately 100-1000 ppm, and PAH were found to be present at 0.1-5% in the same sample (17). The amino- and hydroxy-substituted heterocycles were identified at low levels by utilizing selective detectors during the analysis of fractions in which one of the two functional groups was enriched: FPD for the nitrogen heterocycles and amino-PAC fraction, FPD for the hydroxyl-PAC fractions, ECD for the tertiary nitrogen PAC fraction, and NPD for the hydroxyl and nitrogen-PAC fractions. Compounds were positively identified by comparison of retention data with those of standard compounds and by selected ion mass spectrometry. Figure 2 shows one example: the ECD chromatogram of the PFP-derivatized SRC II HD S-3 fraction. This chromatogram shows only the

PFPP-amide and tertiary nitrogen-containing compounds; the aminophenylpyridines, aminophenylquinolines, and their alkylated derivatives were identified by GC-MS.

All isomers of the aminodibenzothiophenes and the azadibenzothiophenes were assayed for mutagenicity using the Ames test (2,4). The 2- and 3-aminodibenzothiophenes were strongly mutagenic, and their average response was ten to one hundred times greater than the average response of benzo[a]pyrene, while all isomers of the azadibenzothiophenes were inactive. Recently, the microbial mutagenicities of numerous isomeric 3- to 5-ring sulfur heterocycles have been extensively studied (18-20). The presence of a sulfur heteroatom was found to have little effect on mutagenicity. Likewise, compounds containing both nitrogen and sulfur heteroatoms in the rings also demonstrated little mutagenic activity.

Representative PAC structures found in the SRC II HD and the coal tar containing one heteroatom and two heteroatoms are given in Tables 1 and 2, respectively. It is thought that the major reason for the difference between the two samples was auto-catalytic mild hydrogenation in the SRC II process. This would explain the difference in abundance of the amino compounds, hydroxyl PAC, sulfur heterocycles with fusion on only one side of the thiophene ring, 4H-benzo[def]carbazole, etc. The amino and hydroxyl groups in the SRC II HD are thought to be derived from nitrogen and oxygen heterocycles by hydrogenation. The 2- and 3-ring amino-PAH in the SRC II HD were the major components of the amino-PAH fraction, while only the aminonaphthalenes were detected in the coal tar, and then only at a low level (5). Likewise, the aminodibenzothiophenes in the SRC II HD were present as major components, but only the azathiophenic compounds were present in the coal tar (4). Also, the hydroxyphenylthiophenes and the hydroxyphenylbenzothiophenes were more abundant than the hydroxybenzothiophenes and hydroxydibenzothiophenes in the SRC II HD (1). On the other hand, only the hydroxybenzothiophenes and hydroxydibenzothiophenes were present in the coal tar. Figures 3 and 4 show chromatograms of the hydroxyl nitrogen-PAC fractions of both samples. Marked abundances of the hydrogenated compounds, i.e. hydroxyphenylpyridines, was noticeable in the SRC II HD.

Structural similarities between the heteroatom-containing PAC and the PAH were found. Structures of the PAC containing two heteroatoms were similar to those of the PAC containing one heteroatom, and the structures of the PAC containing one heteroatom reflect the parent PAH structures. The relationships between structure and abundance for these compounds are discussed in detail elsewhere (17).

ACKNOWLEDGMENT

This work was supported by the Department of Energy, Office of Health and Environmental Research, Contract No. DE-AC02-79EV10237.

REFERENCES

1. Nishioka, M., Lee, M. L., Kudo, H., Muchiri, D. R., Baldwin, L. J., Pakray, S., Stuart, J. G. and Castle, R. N. Anal. Chem. 1985, 57, 1327.
2. Nishioka, M., Campbell, R. M., West, W. R., Smith, P. A., Booth, G. M., Lee, M. L., Kudo, H. and Castle, R. N. Anal. Chem. 1985, 57, 1868.
3. Nishioka, M., Campbell, R. N., Lee, M. L., Muchiri, D. R., Stuart, J. G. and Castle, R. N. Anal. Chem. 1985, 57, 2211.

4. Nishioka, M., Smith, P. A., Booth, G. M., Lee, M. L., Kudo, H., Muchiri, D. R., Castle, R. N. and Klemm, L. H. Fuel in press.
5. Nishioka, M., Lee, M. L., and Castle, R. N. Fuel submitted.
6. Refer to references cited in (1)-(5).
7. Later, D. W., Lee, M. L., Bartle, K. D., Kong, R. C. and Vassilaros, D. L. Anal. Chem. 1981, 53, 1612.
8. Kuei, J. C., Shelton, J. I., Castle, L. W., Kong, R. C., Richter, B. E., Bradshaw, J. S. and Lee, M. L. J. High Resolut. Chromatogr. Chromatogr. Commun. 1984, 7, 13.
9. Lee, M. L., Kuei, J. C., Adams, N. W., Tarbet, B. J., Nishioka, M., Jones, B. A. and Bradshaw, J. S. J. Chromatogr. 1984, 302, 303.
10. Jones, B. A., Bradshaw, J. S., Nishioka, M. and Lee, M. L. J. Org. Chem. 1984, 49, 4947.
11. Markides, K. E., Nishioka, M., Tarbet, B. J., Bradshaw, J. S. and Lee, M. L. Anal. Chem. 1985, 57, 1296.
12. Markides, K. E., Chang, H.-C., Schregenberger, C. M., Tarbet, B. J., Bradshaw, J. S. and Lee, M. L. J. High Resolut. Chromatogr. Chromatogr. Commun. in press.
13. Nishioka, M. Jones, B. A., Tarbet, B. J., Bradshaw, J. S. and Lee, M. L. J. Chromatogr. submitted.
14. McCann, J. and Ames, B. N. Proc. Natl. Acad. Sci. 1976, 73, 950.
15. Nishioka, M., Chang, H.-C. and Lee, M. L. Env. Sci. Tech. submitted.
16. Nishioka, M., Lee, M. L. and Castle, R. N. Fuel in press.
17. Nishioka, M. and Lee, M. L. Fuel submitted.
18. Pelroy, R. A., Stewart, D. L., Tominaga, Y., Iwao, M., Castle, R. N. and Lee, M. L. Mutat. Res. 1983, 117, 31.
19. Eastmond, D. A., Booth, G. M. and Lee, M. L. Arch. Environ. Contam. Toxicol. 1984, 13, 105.
20. Pelroy, R. A., Stewart, D. L., Lee, M. L., Pratap, R., Tominaga, Y., Iwao, M., Thompson, R. D. and Castle, R. N. Mutation Res. in press.

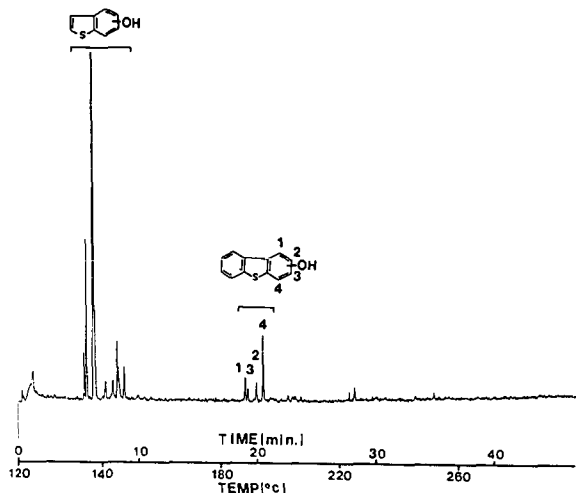


Figure 1. FPD chromatogram of the coal tar A-4 trimethylsilyl derivatized fraction on a 25% biphenyl polysiloxane stationary phase. Conditions: temperature program from 120°C to 265°C at 4°C min⁻¹, after an initial 2-min isothermal period; hydrogen carrier gas at 100 cm s⁻¹.

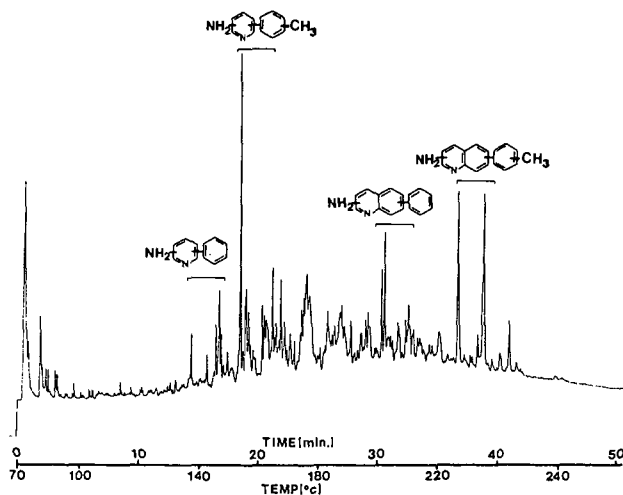


Figure 2. ECD chromatogram of the SRC II HD S-3 PFP derivatized fraction on SE-54. Conditions: temperature program from 70°C to 100°C at 10°C min⁻¹, then from 100°C to 265°C at 4°C min⁻¹ after an initial 2-min isothermal period; helium carrier gas at 50 cm s⁻¹.

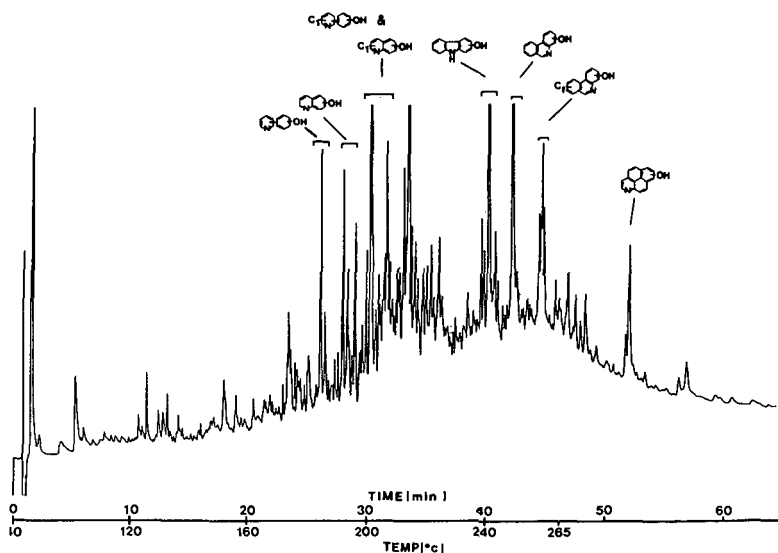


Figure 3. NPD chromatogram of the SRC II HD S-2' fraction on a 25% biphenyl polysiloxane stationary phase. Conditions: temperature program from 40°C to 120°C at 10°C min⁻¹, then from 120°C to 265°C at 4°C min⁻¹, after an initial 2-min isothermal period; helium carrier gas at 50 cm s⁻¹.

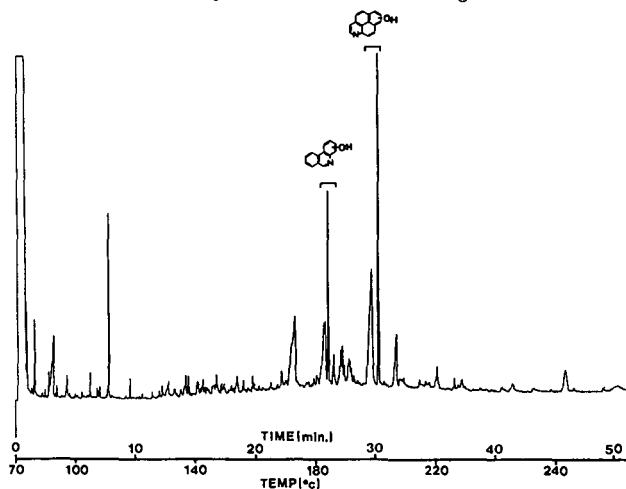


Figure 4. FID chromatogram of the coal tar S-2' fraction on SE-54. Conditions: temperature program from 70°C to 100°C at 10°C min⁻¹, then from 100°C to 265°C at 4°C min⁻¹, after an initial 2-min isothermal period; hydrogen carrier gas at 100 cm s⁻¹.

Table 1. Comparison of the PAC containing one heteroatom identified in the SRC II coal liquid and the coal tar.























































PASH		N-PAC						HPAH	
		2'-PANH		APAH		3'-PANH			
Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II
	-		-	-			-	-	
				-				-	
			-	-			-		-
			-						-
	-		-				-		
									
			-						

Table 2. Comparison of the PAC containing two heteroatoms identified in the SRC II coal liquid and the coal tar.

PANSH / APASH		HPASH		HPANH	
Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II
	-				
		-		-	
-					
	-	-	